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13. SUPPLEMENTARY NOTES

14. ABSTRACT A breast cancer patient with estrogen receptor positive (ER+) invasive ductal carcinoma (IDC) typically has the tumor removed, possibly with nearby lymph nodes, and hormonal therapy is begun. Then a significant decision is made: should the patient receive adjuvant chemotherapy to attack cells that have escaped the tumor? In IDC ER+ patients whose cancer has spread to the lymph nodes the choice is clear and virtually all are treated systemically. However, in the majority of IDC ER+ cases the cancer has not yet spread ("N0"), and the choice is unclear. Current data suggests that about half of patients that are systemically treated would *not* have metastasized, did *not* need to suffer the toxic effects of systemic therapy, and were "overtreated". Hence there is a pressing need to predict who will, and will not, metastasize, to minimize overtreatment ***We hypothesize that SHG F/B is a clinically useful predictor of metastatic outcome.*** In an existing TMA we will answer the following questions: 1) Does F/B predict metastatic outcome in IDC patients? 2) Does F/B predict metastatic outcome in ER+ IDC patients? 3) Does F/B predict metastatic outcome in ER+ IDC patients treated with hormonal, chemo, and/or radiotherapy?

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Introduction: When treating a patient with invasive breast cancer, after surgical resection of the primary tumor the clinician must choose appropriate systemic therapy based upon the predicted metastatic potential of the tumor. For some patients this choice is relatively clear: For example, estrogen receptor negative (ER-) tumors make up approximately 20% of breast tumors, and are a more aggressive subtype that is universally treated with cytotoxic chemotherapy. In the ER+ population, significant strides have been made using adjuvant hormonal therapy alone, such as Tamoxifen, which has a significant, positive effect on patient outcome. Furthermore, in the subset of ER+ patients whose cancer has already metastasized to the lymph nodes (classified as N1, N2, or N3) the choice of treatment is clear and systemic chemotherapy is routinely administered in addition to hormonal therapy. However, in the subset of ER+ patents whose cancer has not metastasized to the lymph nodes (N0) the choice between Tamoxifen alone or Tamoxifen with additional chemotherapy is less clear. Current data suggests that approximately half of patients that are treated with adjuvant chemotherapy would *not* have metastasized, did *not* need to suffer the toxic effects of chemotherapy therapy, and were "overtreated". Hence there is a pressing clinical need to accurately predict exactly who will, and will not, metastasize in the ER+ N0 population in order to minimize the problem of overtreatment. In recent years improvement has been made in outcome prediction, as a function of receptor status, genetic predisposition, morphological features of the tumor, and immunohistochemistry. Specifically, in the case of ER+ breast cancer it has been demonstrated that tumor metastatic potential and response to treatment can be predicted to various degrees of accuracy using gene expression measurements, immunohistochemistry of gene related protein products, and image analysis of cell interactions within the tumor. One example of these methods is "Oncotype DX", a 21-gene test which is the standard of care at our affiliated hospital (Strong Memorial Hospital, Rochester, NY) and assists Dr. Kristin Skinner, the surgical oncologist on our collaborative team, with chemotherapy decisions for ~80% of patients. However it is far from perfect, and 69.5% of patients in the "high risk" category do not get metastases after 10 years. It is also expensive, at more than $4k per test. A commonly used alternative is the conventional information produced by hospital pathology labs, including tumor stage, tumor grade, tumor nodal status, and various molecular markers such as ER, PR, HER2, and Ki-67. These are substantially less expensive than an Oncotype test, but are also less accurate. Consequently, we identified a pressing need to improve the accuracy and cost of metastatic prediction for breast cancer patients.

Our pursuit of this goal began with the realization that the majority of the information derived from genomic methods and conventional pathology analyses focuses on the *cells* within tumors, including their morphological properties and gene expression. Less attention is paid to the extracellular matrix *through which metastasizing cells must travel*. We and others have demonstrated that tumor collagen structure, as measured with the optical process called second harmonic generation (SHG), influences tumor metastasis. This suggests that collagen structure may provide prognostic information about metastasis that is "matrix-focused" and hence complementary, or even superior, to current "cell-focused" genomic methods. Most recently we found that one SHG-based measure of collagen structure in the primary tumor, the SHG forward- to backwards-scattering ratio (F/B), changed significantly with tumor invasiveness (1), consistent with our observation that tumor cells locomote farther in collagen gels manufactured with lower F/B (2). Consequently ***we hypothesized that SHG F/B is a clinically useful predictor of metastatic outcome.*** In a preliminary study in a cohort of 125 ER+ patient samples from the Netherlands, who did not undergo adjuvant hormonal- or chemo- therapy, and who were N0 upon primary tumor excision *we found that F/B measurement in primary tumor samples was a significant prognostic indicator of time to metastasis* (3). This is the very population in which key treatment decisions regarding adjuvant chemotherapy must be made, and for whom improved prognostic information on metastasis is required to reduce overtreatment. F/B was not prognostic in the combined ER+ and ER- IDC N0 population of

221 Dutch patients who received no adjuvant therapy (3). In order to bring this idea closer to the clinic we proposed to repeat that preliminary observation in a second IDC ER+ patient population, based in the U.S., and who underwent adjuvant hormonal therapy, or hormonal and chemotherapy (a much more common scenario than found in the patients of our preliminary study who underwent no adjuvant therapy of any kind). Furthermore we proposed to develop a clinically relevant F/B-based prediction algorithm using separate training and validation sets, etc.

Keywords: metastasis, overtreatment, extracellular matrix, collagen, second harmonic generation

Overall Project Summary: In this **one year** retrospective study we proposed to pursue the Aims described below:

Aim 1. How effectively does F/B predict time to metastasis in ER+ IDC patients that received hormonal therapy?

Aim 1a. Use a training set of F/B values to derive a predictive algorithm using F/B alone, as well as in combination with the Magee score and conventional clinical information.

Aim 1b. Use a validation set of F/B values to test the ability of the F/B algorithm to predict time to metastasis. Test this F/B algorithm against, and in combination with, the predictive power of the Magee score and conventional clinical information.

Aim 1c. Evaluate the ability of the algorithm to also predict response to adjuvant chemotherapy.

Aim 1d. Evaluate the effect of non-randomized study design by repeating 1a and 1b within the subset of patients that did not receive adjuvant chemotherapy.

Aim 2. How effectively does F/B predict time to metastasis in all IDC patients?

Repeat Aims 1a-1d in the entire patient cohort, including all ER+ and ER- patients.

This was specifically to be performed on a pre-existing tissue microarray (TMA) which we had already secured, consisting of multiple samples from patients with IDC N0 and ER+ or ER-, with followup data. Our successful completion of these aims began when we first generated the F/B data for each patient in the TMA. This consists of a blinded user manually focusing on a sample, and generating an F and B image stack. This large number of samples takes quite a bit of time, and as the microscope is a multiuser system one must account for variations in system alignment, etc., between imaging sessions, therefore periodically a calibration sample consisting of free FITC in solution is imaged with the same F and B system (free FITC emits isotropically and therefore has a known constant F/B=1). Images were then analyzed by a blinded user as follows (3): first a maximum intensity projection was performed (this acts as an "autofocus" and reduces the effects of variations in focus between the two-photon focal volume and the thin tissue section), producing a single F and B image for each sample. Then an arbitrary threshold was chosen by a blinded user for all F and all B images (2 thresholds in total) and applied to each F and B image thereby selecting only those pixels that are above threshold (i.e. pixels within collagen fibers). Then the F/B was calculated for each pixel above threshold in both images, and the average of all such pixels was calculated, producing the average F/B of all fibers in a given image. This F/B was adjusted by the F/B of the FITC calibration sample to account for any minor variations in system throughput. Finally, the average of each patient's samples were calibrated. As seen in Figure 1 there was a slight trend in F/B measured as a function of the image number. As Figure 2 reveals a stable FITC calibration correction factor over time (i.e. over imaging session) we concluded that the trend in F/B was real, and due to the distribution of sample

F/B values over the TMA slide. Figure 3 shows a small but statistically significant difference in average F/B of treated US patients versus untreated Dutch patients.

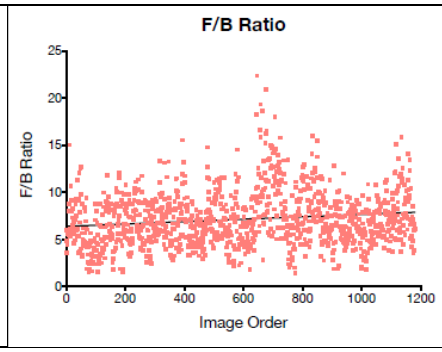

Fig. 1. Display of all sample F/B values reveals a slight but statistically significant trend in F/B values as a function of imaging time.

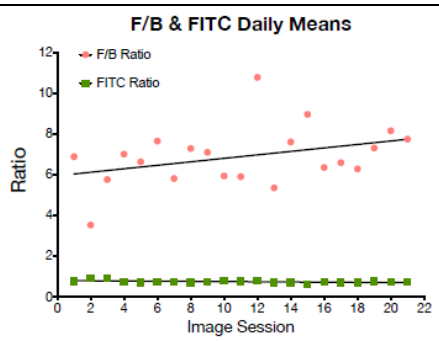

Fig. 2. Evaluation of the FITC calibration factor (green) versus time (i.e. imaging session) reveals no significant trend, suggesting the system was stable over time.

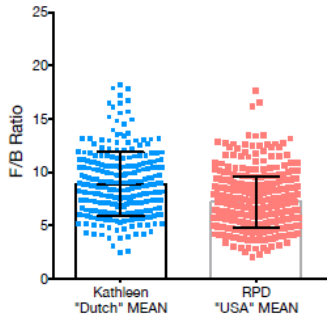

Fig. 3. Comparison of the mean F/B values of patients in the new TMA versus the untreated Dutch data set reveals a small but statistically-significant difference between the two populations (Mann-Whitney test, $p<0.0001$).

Once we had assembled the array of F/B values for all patients in the TMA, the analysis could begin. First we determined the Magee score for each patient based upon the patient records. The Magee score is a low cost surrogate for the OncotypeDX score (we planned to use the Magee score for this very reason). Next we pursued Aims 1a/1b. Preliminary analysis found that there was no statistically significant relationship between F/B and time to metastasis in this data set (Wilcoxon $p=0.47$). This was further confirmed by evaluating multiple predictive models using double cross-validation, essentially dividing the data set into training and validation subsets multiple times, each time selecting a different training and validation subset at random from the overall data set. Interestingly, in this data set patient age and tumor size were *more* predictive than the Magee score. We found that addition of F/B did not significantly improve predictive accuracy (Table 1).

	MSE	Rate	Likelihood
Model 1: Age+Size	0.07688	0.9087	-7.196
Model 2: Age+Size+F/B	0.07677	0.9095	-7.2463
Model 3: Age+Size+RAND	0.07793	0.9035	-7.2962
Model 4: Magee	0.08248	0.9082	-7.8168
Model 5: Magee+F/B	0.08267	0.9076	-7.8682
Model 6: Magee+RAND	0.08451	0.9042	-7.9282

Table 1. Effects of adding different predictors to the accuracy of metastatic prediction. Age is patient age upon first clinical presentation, Size is primary tumor size upon excision, F/B is the second harmonic generation forwards-to backwards scattering ratio, while RAND is a random number between zero and one. MSE represents the mean squared error and is measured as the squared deviation from the predicted probability (i.e. lower is better). Rate represents the rate at which the model correctly predicted the outcome (i.e. higher is better). Likelihood is another measure of predictive accuracy (i.e. higher is better). Interestingly, Age+Size is more predictive than Magee score, but combination of F/B with Age and Size does not significantly improve predictive accuracy.

The comparison between models can be visually observed in Figure 4, where the MSE score for Model 1 is plotted against the MSE score for Model 2 (these were the two best models). An improvement in predictive accuracy would be represented by points lying under the diagonal, but this curve remains with a slope=1.

Next we pursued Aim 1c. To discover if a predictor of time to metastasis can also predict response to chemotherapy, one must compare Kaplan-Meir curves in the presence and absence of chemotherapy. As shown in the Figure 5 cartoon, one looks at the survival characteristics of treated and untreated patients who have differing scores of the candidate predictor. If differing scores segregates the population into groups which react differently to chemotherapy, then the candidate predictor also indicates response to chemotherapy.

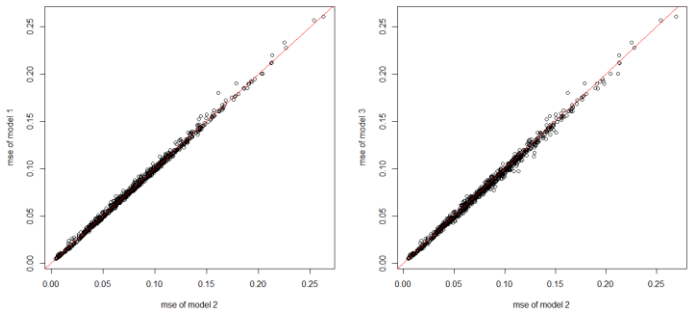

Figure 4. Comparison of MSEs from predictive models 1 and 2 (left: Age+Size versus Age+Size+F/B) and MSEs from predictive models 2 and 3 (right: Age+Size+F/B versus Age+Size+RAND). No significant shift in MSEs is found in either case. The points represent 1000 cross-validated subsamples.

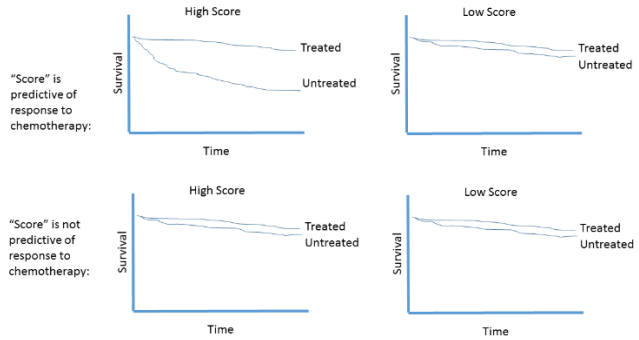

Figure 5. A cartoon illustrating the concept of how a candidate predictor of metastatic outcome ("score") can be evaluated to determine if it also serves to predict response to chemotherapy. In the top case "score" is found to be predictive of response to therapy because high scoring patients are efficaciously treated with chemo, while low scoring patients exhibit no chemo effect. In the lower case "score" is not predictive because high and low scoring patients exhibit the same chemo effect (4).

The results of this analysis are found in condensed fashion in Table 2, where we see a Cox Proportional Hazards analysis of the difference in survival curves due to hormonal treatment (H), radiation (R), and chemotherapy (C) for patients with low F/B, medium F/B, and high F/B (representing a three-column version of the cartoon in Figure 5):

	Low F/B		Medium F/B		High F/B	
	Coeff	P-value	Coeff	P-value	Coeff	P-value
H	-1.5461	0.0291	-0.7731	0.131	-0.1726	0.799
R	-1.3445	0.0529	0.3979	0.535	-0.9277	0.126
C	-0.6868	0.333	1.2505	0.0519	0.8348	0.218

Table 2: Cox Proportional Hazards analysis of difference in survival curves due to hormonal treatment, radiation, and chemotherapy.

As explained in the cartoon of Figure 5, the presence of significant differences in survival curves due to treatment in some F/B regimes AND its absence in others suggests that F/B offers an ability to predict treatment efficacy in this patient cohort.

Next we pursued Aim 1d by exploring the predictive powers of several models (i.e. several different combinations of candidate predictors) in the cohort of patients that did not receive adjuvant chemotherapy using a multivariate linear model. We also evaluated this in the cohort that did not receive adjuvant radiation therapy. As shown in Table 3 (following pages), in the blue columns, addition of F/B as a predictor (rows 4, 7, 8, 10, 11,

12, 14, 15) to other combinations of predictors did not statistically significantly improve the predictive ability of the model, as evidenced by the large p values.

Table 3 (following page). Each row represents the ability of a different predictive model i.e. a specific combination of predictors to predict distant metastasis. The first blue column displays the coefficient of each predictor in the model, and the p value of that predictor, when applied to the cohort that received no adjuvant chemotherapy. The second blue column represents the application of each predictor to the cohort that received no radiation therapy. The fact that F/B did not statistically significantly improve outcome prediction in this cohort is evidenced by the large p values of that predictor in each model (red text).

	Table 3	No C therapy, ER+			C therapy, ER+			No R therapy, ER+			R therapy, ER+		
	Model	β	p	R^2	β	p	R^2	β	p	R^2	β	p	R^2
1	Distant~ age	0.001	0.734	0.001	-0.002	0.381	0.006	0	0.901	0	-0.002	0.219	0.009
2	Distant ~ magee	0.014	0.005	0.078	0.003	0.497	0.004	0.002	0.746	0.002	0.009	0.014	0.039
3	Distant ~ size	0.114	0	0.235	0.046	0.025	0.042	0.037	0.23	0.025	0.081	0	0.134
4	Distant ~ F/B	0.005	0.633	0.002	0.003	0.81	0	-0.007	0.687	0.003	0.007	0.475	0.003
5	Distant ~ age + size	0.114	0	0.235	0.046	0.025	0.042	0.037	0.23	0.025	0.081	0	0.134
6	Distant ~ age + magee	-0.001age +0.014magee	0.996 0.005	0.079	-0.005age +0.003magee	0.091 0.465	0.032	-0.001age +0.001magee	0.596 0.780	0.007	-0.003age +0.009magee	0.084 0.020	0.054
7	Distant ~ age + F/B	0.001age +0.005FB	0.735 0.644	0.003	-0.002age +0.003FB	0.383 0.844	0.007	0AGE -0.007FB	0.902 0.696	0.003	-0.002AGE +0.007FB	0.219 0.427	0.013
8	Distant~ size + F/b	0.114size +0FB	0 0.97	0.235	0.046SIZE +0.002FB	0.025 0.86	0.042	0.036SIZE -0.006FB	0.234 0.709	0.027	0.08SIZE 0.004FB	0 0.631	0.135
9	Distant ~ size + magee	0.092size +0.008magee	0 0.105	0.227	0.032size 0.002magee	0.177 0.643	0.019	0.024size +0magee	0.438 0.968	0.012	0.073size +0.006magee	0 0.104	0.125
10	Distant ~ magee + F/B	0.014magee +0.008F.B	0.005 0.378	0.086	0.003magee +0.008F.B	0.499 0.561	0.008	0.002magee +0.001F.B	0.748 0.933	0.002	0.009MAGEE +0.011	0.014 0.226	0.049
11	Distant ~ magee + F/B + age	0.014magee +0.009F.B -0.001age	0.005 0.380 0.717	0.087	0.003magee +0.007F.B -0.005AGE	0.495 0.557 0.093	0.035	0.002magee +0.001F.B -0.001AGE	0.750 0.933 0.620	0.007	0.009magee+ 0.013F.B -0.003age	0.014 0.224 0.107	0.065
12	Distant ~ magee + F/B + size	0.008magee +0.004F.B +0.091SIZE..cm.	0.002 0.340 0	0.229	0.002magee +0.006F.B +0.031SIZE	0.498 0.560 0.230	0.022	0MAGEE +0.001f.b +0.024size	0.749 0.933 0.486	0.012	0.006magee +0.008F.B 0.071SIZE	0.011 0.207 0	0.129
13	Distant ~ magee + age + size	0.008magee -0.002age +0.096size	0.002 0.741 0	0.236	0.002magee -0.005age +0.036size	0.491 0.086 0.150	0.051	0magee -0.001age +0.023size	0.749 0.615 0.502	0.016	0.005magee -0.003age +0.076size	0.1 0.111 0	0.146
14	Distant ~ F/B + age + size	0F.B -0.001AGE +0.117SIZE	0.588 0.717 0	0.238	0.001F.B -0.003AGE +0.048SIZE	0.787 0.399 0.020	0.052	-0.006F.B +0AGE +0.037SIZE	0.688 0.926 0.239	0.028	0.005F.B- 0.003AGE +0.081SIZE	0.432 0.176 0	0.148
15	Distant ~ magee + F/B + age + size	0.008magee +0.005F.B -0.002age +0.095size	0.002 0.340 0.693 0	0.239	0.002magee+ 0.005F.B- 0.005age +0.035size	0.493 0.555 0.091 0.165	0.053	0magee +0F.B -0.001AGE 0.023SIZE	0.751 0.934 0.622 0.508	0.016	0.005magee+ 0.009F.B -0.003age +0.074size	0.01 0.203 0.092 0	0.152

Next we pursued Aim 2 by first evaluating the predictive ability of models incorporating F/B using a multivariate linear model, but this time applying the analysis to the entire ER+ and ER- cohort. As shown in Table 4 and 5 (following pages), addition of F/B to the predictive models did not provide additional predictive accuracy when applied to the combined ER+ and ER- cohort that received both chemo and radiotherapy (last peach column, Table 4). Nor did it provide additional predictive accuracy when applied to the combined ER+ and ER- cohort that received no chemotherapy (second peach column, Table 5) or no radiotherapy (last peach column, Table 5). However, it did provide some degree of predictive ability when applied to the combined ER+ and ER- cohort that received no adjuvant chemo- nor radiotherapy (first blue column Table 4, green boxes and green p values), as well as in the subgroup of the combined ER+ and ER- cohort that received only radiation therapy (first peach column, Table 4, green boxes and green p values).

Table 4 and 5 (following page). Each row represents the ability of a different predictive model i.e. a specific combination of predictors to predict distant metastasis. The columns represent different combinations of therapies that the subgroup was subjected to. The presence of a low p value indicates that the corresponding predictor has value in predicting distant metastasis in the given treatment subgroup of the combined ER+ and ER- cohort.

	Table 4	No therapy, ER+& ER-			Only R therapy, ER+& ER-			Only C therapy, ER+& ER-			Both CR therapy, ER+& ER-		
	Model	β	p	R^2	β	p	R^2	β	p	R^2	β	p	R^2
1	Distant~ age	0	0.955	0	-0.001	0.496	0.004	-0.004	0.343	0.017	-0.004	0.171	0.014
2	Distant ~ magee	0.013	0.03	0.144	0.002	0.458	0.006	0.005	0.222	0.034	0.004	0.223	0.013
3	Distant ~ size	0.035	0.5	0.012	0.117	0	0.267	-0.009	0.754	0.002	0.058	0	0.104
4	Distant ~ F/B	-0.049	**0.072**	0.09	0.004	0.577	0.003	0.017	0.404	0.014	-0.003	0.814	0
5	Distant ~ age + size	0age+ 0.035size	0.955 0.506	0.012	-0.003age +0.122size	0.422 0	0.287	-0.004age -0.009size	0.394 0.752	0.017	-0.004age +0.058size	0.177 0	0.177
6	Distant ~ age + magee	-0.003age +0.013magee	0.552 0.029	0.157	0age+ 0.002magee	0.868 0.463	0.006	-0.002age +0.006magee	0.744 0.207	0.04	-0.003age 0.004magee	0.252 0.261	0.023
7	Distant ~ age + F/B	0.002AGE- 0.051FB	0.910 **0.068**	0.095	-0.001AGE +0.005FB	0.524 0.540	0.007	-0.004AGE +0.014FB	0.308 0.495	0.03	-0.003AGE -0.003FB	0.238 0.816	0.012
8	Distant~ size + F/b	0.036size -0.048fb	0.434 **0.06**	0.104	0.12size +0fb	0 0.943	0.275	-0.009size +0.016fb	0.712 0.435	0.015	0.055size -0.002fb	0 0.898	0.1
9	Distant ~ size + magee	-0.01size+ 0.013magee	0.983 0.032	0.145	0.129size+ 0.001magee	0 0.608	0.345	-0.039size +0.007magee	0.58 0.13	0.06	0.055size 0.003magee	0.001 0.438	0.105
10	Distant ~ magee + F/B	0.015MAGEE -0.025fb	0.004 0.353	0.265	0.002magee +0.014fb	0.471 0.142	0.032	0.006magee 0.024fb	0.236 0.267	0.062	0.003magee 0.001fb	0.397 0.963	0.007
11	Distant ~ magee + F/B + age	0.015MAGEE -0.023FB -0.002AGE	0.005 0.361 0.689	0.269	0.002MAGEE +0.014FB 0AGE	0.474 0.144 0.860	0.032	0.006MAGEE +0.023FB -0.002AGE	0.242 0.273 0.735	0.065	0.003MAGEE 0.001FB -0.003AGE	0.397 0.963 0.339	0.016
12	Distant ~ magee + F/B + size	0.015magee -0.025fb -0.002size	0.005 0.362 0.964	0.265	0.001magee 0.008fb 0.13size	0.378 **0.073** 0	0.363	0.007magee 0.02fb -0.034size	0.238 0.269 0.375	0.08	0.001magee +0fb +0.053size	0.376 0.962 0.002	0.099
13	Distant ~ magee + age + size	0.013magee -0.003age -0.007size	0.034 0.496 0.898	0.158	0.001magee -0.003age +0.134size	0.361 0.858 0	0.361	0.008magee -0.002age -0.039size	0.226 0.622 0.297	0.065	0.002magee -0.003age 0.054size	0.203 0.275 0.001	0.113
14	Distant ~ F/B + age + size	-0.05FB +0.002AGE +0.034size	**0.078** 0.652 0.492	0.108	0.001fb -0.003age 0.125size	0.511 0.417 0	0.295	0.013fb -0.004age -0.01size	0.422 0.416 0731	0.029	-0.002fb -0.004age +0.056size	0.831 0.230 0	0.113
15	Distant ~ magee + F/B + age + size	0.015magee -0.023FB -0.002AGE -0.001SIZE	0.006 0.369 0.694 0.988	0.269	0.001MAGEE 0.009FB -0.003AGE 0.135SIZE	0.375 **0.071** 0.827 0	0.378	0.008MAGEE 0.018FB -0.002AGE -0.035SIZE	0.243 0.274 0.736 0.368	0.084	0.001magee +0FB+- 0.003AGE +0.053SIZE	0.376 0.962 0.318 0.002	0.108

	Table 5	C therapy, ER+ & ER -			No C therapy, ER+ & ER -			R therapy, ER+ & ER -			No R therapy, ER+ & ER -		
	Model	β	p	R^2	β	p	R^2	β	p	R^2	β	p	R^2
1	Distant~ age	-0.01	0.619	0.002	-0.004	0.09	0.015	-0.001	0.745	0.001	-0.004	0.008	0.027
2	Distant ~ magee	0.008	0.004	0.064	0.004	0.194	0.011	0.007	0.051	0.049	0.005	0.037	0.022
3	Distant ~ size	0.095	0	0.132	0.043	0.001	0.054	-0.001	0.959	0	0.072	0	0.15
4	Distant ~ F/B	-0.006	0.44	0.002	0.833	0.833	0	-0.009	0.557	0.004	-0.001	0.91	0
5	Distant ~ age + size	-0.002age +0.099size	0.593 0	0.14	-0.004age+ 0.043size	0.107 0.001	0.068	-0.001age- 0.002size	0.808 0.949	0.001	-0.003age+ 0.07size	0.005 0	0.168
6	Distant ~ age + magee	-0.002age+ 0.008magee	0.496 0.004	0.069	-0.003age +0.004magee	0.235 0.209	0.019	-0.001age +0.007magee	0.681 0.055	0.05	-0.003age +0.004magee	0.042 0.069	0.036
7	Distant ~ age + F/B	0AGE -0.006FB	0.720 0.463	-0.004age +0.002FB	-0.001AGE -0.010FB	0.115 0.879	0.014	-0.001age -0.01FB	0.667 0.548	0.06	-0.004AGE +0.001FB	0.017 0.932	0.024
8	Distant~ size + F/b	0.098size -0.008FB	0.000 0.282	0.152	0.04SIZE +0.003FB	0.003 0.764	0.049	0SIZE- 0.01FB	0.991 0.555	0.004	0.070SIZE +0FB	0 0.971	0.148
9	Distant ~ size + magee	0.082size +0.006magee	0.000 0.018	0.16	0.04size +0.002magee	0.005 0.396	0.054	-0.045size+ 0.01magee	0.550 0.018	0.077	0.070size +0.003magee	0 0.161	0.161
10	Distant ~ magee + F/B	0.009magee +0.006FB	0.001 0.499	0.093	0.003magee 0.008FB	0.342 0.515	0.009	0.009MAGEE +0.004FB	0.02 0.795	0.073	0.004magee +0.007FB	0.092 0.405	0.019
11	Distant ~ magee + F/B + age	0.009magee +0.007FB -0.001age	0.001 0.5 0.568	0.096	0.003magee +0.007FB -0.003AGE	0.341 0.515 0.293	0.017	0.009magee +0.004FB -0.001age	0.021 0.796 0.756	0.074	0.004magee +0.008FB -0.003AGE	0.091 0.403 0.117	0.032
12	Distant ~ magee + F/B + size	0.008magee +0.003FB 0.083SIZE	0.001 0.475 0	0.196	0.002magee +0.008FB +0.038SIZE	0.333 0.507 0.014	0.051	0.011magee +0.003FB -0.044SIZE	0.019 0.793 0.135	0.101	0.002magee +0.006FB +0.068SIZE	0.07 0.37 0	0.159
13	Distant ~ magee + age + size	0.006magee -0.003age +0.087size	0.003 0.431 0	0.172	0.002magee -0.003age +0.04size	0.185 0.245 0.009	0.062	0.009magee -0.001age -0.045size	0.051 0.769 0.139	0.078	0.003magee -0.002age 0.069size	0.024 0.061 0	0.171
14	Distant ~ F/B + age + size	-0.008fb -0.002age 0.101size	0.405 0.765 0	0.157	0.003fb -0.004age +0.041size	0.814 0.129 0.003	0.064	-0.01fb -0.001age -0.001size	0.557 0.710 0.972	0.006	0.001fb -0.003age 0.068size	0.914 0.01 0.000	0.164
15	Distant ~ magee + F/B + age + size	0.008magee +0.004fb -0.002age +0.086size	0.001 0.474 0.511 0	0.205	0.001magee 0.008fb -0.003age 0.038size	0.333 0.507 0.284 0.013	0.058	0.011magee 0.003fb -0.001age -0.045size	0.02 0.794 0.753 0.132	0.103	0.002magee +0.006fb -0.002age 0.068size	0.069 0.368 0.092 0	0.168

Key Research Accomplishments

In this one-year project we accomplished the following:

1. We determined that F/B when added to other predictors does not significantly improve accuracy of predicting time to metastasis in this cohort of ER+ IDC patients that received hormonal therapy.
2. We determined that F/B when added to other predictors does not significantly improve accuracy of predicting time to metastasis in the subset of these ER+ IDC patients that received hormonal therapy but who did not receive chemotherapy.
3. We determined that F/B does offer some ability to predict response to hormonal, radio-, and chemotherapy in this patient set.
4. We determined that F/B when added to other predictors does not significantly improve accuracy of predicting time to metastasis in a mixed cohort of ER+ and ER- IDC patients that received both chemo and radiotherapy, as well as those that received no chemotherapy, and those that received no radiotherapy.
5. We determined that F/B does provide a degree of improved predictive ability when combined with other predictors and applied to the subgroup of the combined ER+ & ER- cohort that received neither radio- nor chemotherapy, as well as the subgroup that received only radiation therapy.

Conclusions

The fact that F/B is correlated with metastatic outcome in untreated patients (3) but not in these tamoxifen treated patients is an interesting result and could either be a function of these particular data sets or an indicator of a key role for the estrogen receptor in the relationship between collagen microstructure (on which F/B reports) and the metastatic process. The indications that F/B offers some ability to predict response to hormonal, radio, and chemotherapy in this patient set warrants further study and development.

Publications, Abstracts, and Presentations

We have not yet presented the results of this one-year study.

Reportable Outcomes

N/A

Other Achievements

N/A

References

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Appendix 1

List of personnel receiving pay from the research effort:

Edward Brown
Peter Salzman
Ryan Dawes
Ziji Yu
Kristin Skinner
Ping Tang